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(74) Agent: REINHOLD COHN AND PARTNERS; P.O.Box
4060, 61040 Tel Aviv (IL).

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(71) Applicant (*for all designated States except US*):
CAN-FITE BIOPHARMA LTD. [IL/IL]; 10 Bareket
Street, 49170 Petach Tikva (IL).

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(72) Inventor; and

(75) Inventor/Applicant (*for US only*): **FISHMAN, Pnina**
[IL/IL]; 19 Asher Barash Street, 46365 Herzliya (IL).

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(54) Title: A3AR AGONISTS FOR THE TREATMENT OF INFLAMMATORY ARTHRITIS

(57) Abstract: The present invention concerns a method for the treatment of inflammatory arthritis, and in particular rheumatoid arthritis, by administering to the subject specific low dosages of N6- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) and 2-chloro-N6- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (CL-IB-MECA).

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A3AR AGONISTS FOR THE TREATMENT OF INFLAMMATORY ARTHRITIS

FIELD OF THE INVENTION

This invention relates to the field of therapeutics and in particular relates to the treatment of arthritis.

PRIOR ART

- 5 The following is a list of prior art, which is considered to be pertinent for describing the state of the art in the field of the invention. Acknowledgement of these references herein will be made by indicating the number from their list below within brackets.
- 10 (1) Olah M.E. and Stiles G.L. The role of receptor structure in determining adenosine receptor activity, *Pharmacol. Ther.*, **85**:55-75 (2000);
- (2) Poulsen S.A. and Quinn R.J., Adenosine receptors: new opportunities for future drugs. *Bioorg. Med. Chem.*, **6**:619-641 (1998);
- (3) Fang X. *et al.* Phosphorylation and inactivation of glycogen synthase kinase
- 15 3 by protein kinase A., *Proc. Natl. Acad. Sci. USA*, **97**:11960-11965 (2000);
- (4) Fishman, P., *et al.*, Involvement of Wnt Signaling Pathway in IB-MECA Mediated Suppression of Melanoma Cells, *Oncogene* **21**:4060-4064 (2002);
- (5) Ferkey, D.M., and Kimelman, D. GSK-3: New Thoughts on an Old Enzyme, *Dev. Biol.*, **225**:471-479 (2000);

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- (6) Bonvini, P., *et al.* Nuclear beta-catenin displays GSK-3 β - and APC-independent proteasome sensitivity in melanoma cells, *Biochim. Biophys. Acta.*, **1495**:308-318 (2000);
- (7) Olah, M.E. and Stiles, G.L, The role of receptor structure in determining adenosine receptor activity, *Pharmacol. Ther.*, **85**:55-75 (2000);
- (8) Szabo C., *et al.* Suppression of macrophage inflammatory protein (MIP)-1 α producing and collagen induced arthritis by adenosine receptor agonists., *British Journal of Pharmacology*, **125**:379-387 (1998);
- (9) U.S. 5,773,423.

10 BACKGROUND OF THE INVENTION

A3 adenosine receptors belong to the family of the Gi-protein associated cell surface receptors. Receptor activation leads to its internalization and the subsequent inhibition of adenylyl cyclase activity, cAMP formation and protein kinase A (PKA) expression, resulting in the initiation of various signaling pathways ^(1,2).

15 PKA contains a catalytic subunit PKAc which dissociates from the parent molecule upon activation with cAMP. Recent studies have demonstrated that PKAc phosphorylates and inactivates the enzyme glycogen synthase kinase 3 β (GSK-3 β) ⁽³⁾.

Recently, it has been shown that 1-deoxy-1-[6[[[(3-iodophenyl)methyl] amino]-9H-purine-9-yl]-N-methyl β -D-ribofuranuronamide (IB-MECA) disclosed

20 in U.S. 5,773,423 incorporated herein by reference, a stable agonist to A3AR, alters the expression of GSK-3 β and β -catenin, key components of the Wnt signaling pathway. Consequently it led to the inhibition of the expression of the cell cycle progression genes, c-myc and cyclin D1 ⁽⁴⁾.

25 Szabo *et al.*, ⁽⁸⁾ reported that IB-MECA suppresses the production of MIP-1 α in macrophages in a dose dependent manner, and was shown to inhibit, also in a dose dependent manner, the production of the cytokines IL-1, IL-2, IL-6 as well as NO. According to this publication, administration of 0.5 mg/kg of IB-MECA a day

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reduced the severity of joint inflammation in a model of collagen-induced arthritis in mice.

Rheumatoid arthritis is a common rheumatic disease, affecting more than two million people in the United States alone. The disease is three times more prevalent in women as in men but afflicts all races equally. The disease can begin at any age, but most often starts between the ages of forty and sixty. In some families, multiple members can be affected, suggesting a genetic basis for the disorder. The cause of rheumatoid arthritis is unknown. Even though infectious agents such as viruses, bacteria, and fungi have long been suspected, none has been proven as the cause. It is suspected that certain infections or factors in the environment might trigger the immune system to attack the body's own tissues, resulting in inflammation in various organs of the body. Regardless of the exact trigger, the result is an immune system that is geared up to promote inflammation in the joints and occasionally other tissues of the body. Lymphocytes are activated and cytokines, such as tumor necrosis factor/TNF and interleukin-1/IL-1 are expressed in the inflamed areas.

The clinical expression of rheumatoid arthritis is manifested by chronic inflammation of the joints, the tissue surrounding the joints such as the tendons, ligaments, and muscles, as well as other organs in the body such as the eyes. The inflammation process of causes swelling, pain, stiffness, and redness in the joints. In some patients with rheumatoid arthritis, chronic inflammation leads to the destruction of the cartilage, bone and ligaments causing deformity of the joints.

SUMMARY OF THE INVENTION

The invention is described in the following SUMMARY with reference to a therapeutic method for the treatment of inflammatory arthritis. It should be noted that in addition to said therapeutic method, also encompassed within the present invention is an oral pharmaceutical composition for the treatment of inflammatory arthritis that comprises an effective amount of the active agents as defined bellow

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and a carrier pharmaceutically acceptable for oral administration; as well as the use of said active agent for the preparation of a pharmaceutical composition for oral administration to a subject suffering of inflammatory arthritis and being in need for an anti-inflammatory treatment. As will be appreciated, the effective amount in the pharmaceutical composition will depend on the intended therapeutic regiment and the desired therapeutic dose. By way of example were the dose is 1 mg per day and the desired administration regiment is once daily administration, the amount of active agent in the pharmaceutical composition will be 1 mg. where it is intended to divide this daily dose in 2 daily administrations, the amount of the active agent in the pharmaceutical composition will be 0.5 mg.

In the following, the term "anti-inflammatory" will be used to denote the disease modifying effect of IB-MECA or Cl-IB-MECA in alleviating the inflammatory response in inflammatory arthritis. The anti-inflammatory response may be determined on the basis of histological parameters, the extent of swollen and tender joints, motility parameters, reduction in pain, a number of different overall performance scoring systems, etc.

The present invention is based on the surprising finding that oral administration of specific A3-receptor agonists, N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (Cl-IB-MECA) alleviated symptoms of inflammatory arthritis at doses which are lower than those previously described, particularly by Szabo *et. al* (8).

The above findings were corroborated by clinical data showing that oral administration of low doses of IB-MECA to humans led to improvements in some of the disease manifestations in rheumatoid arthritis patients.

In the following, unless otherwise indicated, dosages are indicated in weight/Kg meaning to denote weight of administered agent (IB-MECA or Cl-IB-MECA) per kilogram of body of weight in each administration: mg/Kg and microgram/Kg denoting, respectively milligrams of administered agent and

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micrograms of administered agent per kilogram of body weight of the treated subject.

It was also shown that the anti-inflammatory effect of these two drugs appears to be manifested with a surprising dose dependency relationship that is different than might have been expected from the prior art. In particular, it was shown that a potent anti-inflammatory activity is observed over a range of dosages without any dose clear correlation between the dose and the anti-inflammatory effect. Thus, for example, in some animal experiments an oral administration of a dose of 10 microgram/Kg had a stronger anti-inflammatory effect than a dose of 100 microgram/Kg; in some other experiments these two doses had a response that was essentially the same. Typically, pharmaceutically active substances have a classic dose-dependent effect so that the higher the dose the more pronounced the is the therapeutic effect, with the highest dose limitation being usually dictated by undesired side effects (toxicity) that become evident in higher doses. Such a classical dose-response behavior was previously reported for IB-MECA in the suppression of MIP-1 alpha production by Szabo *et. al* ⁽⁸⁾.

A clinician or an investigator wishing to administer IB-MECA or Cl-IB-MECA to a subject as a drug for the treatment of inflammatory arthritis or develop these drugs as a therapy for such disease would have concluded from the prior art dose-response relationship that a dose that is higher than 0.5 milligram/Kg and possibly even much higher, would be suitable for such treatment. Against this, according to the unexpected findings of the present invention, it has been demonstrated that the effect of IB-MECA and Cl-IB-MECA in the alleviation of arthritis occurs with a different dose response curve as could have been envisaged based on the prior art, as also pointed out above. According to the invention, the dose dependent curve features a very strong effect at dosages that are considerably lower than the lowest effective dose reported by Szabo *et. al*, with a very marked effect seen at dosages as low as 25 times lower than lowest one in the Szabo publication. This is particularly so seeing that the administration accordance with

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the prior art, and particularly the said Szabo publication were intraperitoneal while the administration according to the invention is oral in which case the plasma level is limited by absorption.

These findings pave the way to the development of a therapeutic regiment
5 were effective treatment of arthritis can be achieved by administering low oral doses of IB-MECA or Cl-IB-MECA with significantly lower risk of undesired side effects.

Thus, the present invention concerns, by one embodiment, a method for the treatment of inflammatory arthritis (IA) in a human subject, comprising: orally
10 administering to an individual in need of such treatment an effective amount of N⁶-(3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶-(3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (CL-IB-MECA);

wherein the effective amount is an amount which is preferably less than the maximal tolerated dosage (MTD) and is an amount in a range between D_X and D_Y;
15 D_X being an amount lower than D_{MAX} and D_Y being an amount higher than D_{MAX}; D_{MAX} being an amount that yields a maximal therapeutic effect; both D_X and D_Y yield a therapeutic effect that is substantially less than that obtained at D_{MAX}.

The term "*therapeutic effect that is substantially less*" refers to the fact that one or more of the beneficial effects mentioned bellow in reference to the definition
20 of "*treatment*" (decreased swelling, decreased pain, improved motility, slowing of the progression of the disease, increase in the time period of the remission between acute attacks of the disease, decrease in the time period of the acute attack, prevention of the deterioration of the joints etc) is substantially less than that obtained at D_{MAX}. The term "*substantially less*" refers preferably to a therapeutic
25 effect in the treatment of inflammatory arthritis (IA) that is at least about 50% of that that can be achieved with D_{MAX}. As can be appreciated, D_{MAX} and accordingly also D_X and D_Y, may depend on the age, gender, overall health condition, co-administered drugs and other factors. A preferred dose is D_{MAX} or a dose close to it. As may be appreciated, in practice, the effective dose may be set as an average, for

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example based on dose-finding clinical studies. The design of such studies is a routine undertaking of those versed in the art of clinical drug development.

The term: "*inflammatory arthritis*" (IA) refers in the context of the present invention to chronic inflammation, (regardless of the cause but typically due to an autoimmune process that affects the joints), in the tissue around the joints, such as the tendons, ligaments, and muscles, as well as other organs in the body. This term includes rheumatoid arthritis, psoriatic arthritis, IBD-associated arthritis, reactive arthritis, vasculities and SLE. A preferred therapy target in accordance with the invention is rheumatoid arthritis.

10 The term "*treatment*" in the context of the present invention refers to any improvement in the clinical symptoms of the disease, as well as any improvement in the well being of the patients, in particular an improvement manifested by at least one of the following: decreased swelling and tenderness of the joints, decrease in pain in the joints, improved motility, slowing of the deterioration of the joints and the surrounding tissue, increase in the remission period between acute disease attacks; decrease in the time length of the acute attack; prevention of the onset of severe disease, etc.

The effective amount in accordance with the present invention is defined as an amount that is between DX and DY, as defined above. Additionally, this amount should also be less than the maximal tolerated dosage (MTD).

The term "*maximal tolerated dosage*" or "*MTD*" refers to the highest dosage of the active substance that most people can tolerate without side effects. In accordance with clinical trials the maximal tolerated dose of IB-MECA was found to be a dose of about 5 mg (about 70 microgram/Kg; calculated on the basis of average individual human weight of 70 Kg.) for once daily administration and a dose of about 4 mg (about 57 microgram/Kg) for twice daily administration.

The "*effective amount*" can be readily determined, in accordance with the invention, by administering to a plurality of tested subjects various amounts of the active agent and then plotting the physiological response (for example an integrated

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"*arthritic index*" combining several of the therapeutically beneficial effects) as a function of the amount. The amount above which the therapeutic beneficial effects begin to decrease (but is still lower than the MTS) is the "*effective amount*". Due to statistical distribution typically the "*effective amount*" is not a single parameter 5—but a range of parameters.

In murine the effective amount is typically less than about 400 microgram/Kg. A typical dose would be in the range of about 1 microgram/Kg to about 200 microgram/Kg, with a preferred dose being in the range of about 5 microgram/Kg to about 150 microgram/Kg. The corresponding effective amount in 10 human will be an equivalent amount to that observed in murine, which may be determined in a manner as explained bellow.

The present invention thus concerns, by another embodiment, a method for the treatment of inflammatory arthritis (IA) in a human subject, comprising orally administering to a mammal in need of such treatment an effective amount of N⁶-(3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶-(3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (CL-IB-MECA), wherein the 15 effective amount is an amount which the human equivalent of a murine dose of 0.001 mg/Kg to 0.4 mg/Kg administered once or more, preferably twice a day.

The term "*human equivalent*" refers to the dose that produces in human the 20 same effect as featured when a dose of 0.001-0.4 mg/Kg of IB-MECA or CL-IB-MECA is administered to a mouse or a rat. As known, this dose depends and may be determined on the basis of a number of parameters such as body mass, body surface area, absorption rate of the active agent, clearance rate of the agent, rate of metabolism and others.

25 The human equivalent may calculated based on a number of conversion criteria as explained bellow; or may be a dose such that either the plasma level will be similar to that in the murine following administration at a dose as specified above; or a dose that yields a total exposure (namely area under the curve – AUC –

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of the plasma level of said agent as a function of time) that is similar to that in murine at the specified dose range.

It is well known that an amount of X mg/Kg administered to rats can be converted to an equivalent amount in another species (notably humans) by the use of one of possible conversions equations well known in the art. Examples of conversion equations are as follows:

Conversion I:

Species	Body Wt. (Kg)	Body Surf. Area (m ²)	Km Factor
Mouse	0.2	0.0066	3.0
Rat	0.15	0.025	5.9
Human Child	20.0	0.80	25
Adult	70.0	1.60	37

10

Body Surface area dependent Dose conversion: Rat (150g) to Man (70 Kg) is 1/7 the rat dose. This means that in the present case 0.001-0.4 mg/Kg in rats equals to about 0.14-56 microgram/Kg in humans; assuming an average weight of 70 Kg, this would translate into an absolute dosage of about 0.01 to about 4 mg.

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Conversion II:

The following conversion factors: Mouse = 3, Rat = 67. Multiply the conversion factor by the animal weight to go from mg/Kg to mg/m² for human dose equivalent.

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Species	Weight (Kg)	BSA (m ²)
Human	70.00	1.710
Mouse	0.02	0.007
Rat	0.15	0.025
Dog	8.00	0.448

According to this equation the amounts equivalent to 0.001-0.4 mg/Kg in rats for humans are 0.16-64 µg/Kg ; namely an absolute dose for a human weighing about 70 Kg of about 0.011 to about 4.4 mg, similar to the range indicated in

5 Conversion I.

Conversion III:

Another alternative for conversion is by setting the dose to yield the same plasma level or AUC as that achieved following administration to an animal. Based on measurement made in mice following oral administration of IB-MECA and based on such measurements made in humans in a clinical study in which IB-MECA was given to healthy male volunteers it was concluded that a dose of 1 microgram/Kg – 400 microgram/KG in mice is equivalent to a human dose of about 0.14 – 57 microgram/Kg, namely a total dose for a 70 Kg individual of 0.01

10 – 4 mg. This is again similar to the dosages calculated according to Conversion I and II.

Based on the above conversion methods, the preferred dosage range for IB-MECA and CI-IB-MECA would be less than 4 mg, typically within the range of about 0.01 to about 2 mg (about 0.14 – 28 micrograms/Kg, respectively) and

20 desirably within the range of about 0.1 to 1.5 mg (about 1.4 – 21 micrograms/Kg, respectively). This dose may be administered once, twice or at times several times a day. Human studies showed (data not shown herein) that the level of IB-MECA decays in the human plasma with a half life of about 8-10 hours, as compared to a half life of only 1.5 hours in mice, in case of multiple daily administration,

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correction in the dosages for accumulative effects needs to be made at times (a subsequent dose is administered before the level of a previous one was decayed and thus there is a build-up of plasma level over that which occurs in a single dose. On the basis of said human trials twice daily administration appears to be a preferred 5—administration regiment. However this does not rule out other administration regiments.

The present invention discloses for the first time clinical trials in humans showing the effectiveness of IB-MECA, in a specific dosage range, in the treatment of rheumatoid arthritis. In this human study IB-MECA was administered to patients 10 in dose ranging between 0.1 to 4 mg twice daily.

The administration of said agent to a patient may be together with a pharmaceutically carrier acceptable for oral administration.

By the term "*pharmaceutically acceptable carrier*" it is meant any one of inert, non-toxic materials, which do not react with the IB-MECA or Cl-IB-MECA 15 and which can be added to oral formulations as diluents or carriers or to give form or consistency to the formulation. The formulation may be in the form of a pill, capsule, in the form of a syrup, an aromatic powder, and other various forms. The carrier is selected at times based on the desired form of the formulation. The carrier may also at times have the effect of the improving the delivery or penetration of the 20 active ingredient to the target tissue, for improving the stability of the drug, for slowing clearance rates, for imparting slow release properties, for reducing undesired side effects etc. The carrier may also be a substance that stabilizes the formulation (e.g. a preservative), for providing the formulation with an edible flavor, etc. The carriers may be any of those conventionally used and is limited only 25 by chemical-physical considerations, such as solubility and lack of reactivity with IB-MECA or CL-IB-MECA, and by the route of administration. The carrier may include additives, colorants, diluents, buffering agents, disintegrating agents, moistening agents, preservatives, flavoring agents, and pharmacologically compatible carriers. In addition, the carrier may be an adjuvant, which, by

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definition are substances affecting the action of the active ingredient in a predictable way. Typical examples of carriers include (a) liquid solutions, where an effective amount of the active substance is dissolved in diluents, such as water, saline, natural juices, alcohols, syrups, etc.; (b) capsules (e.g. the ordinary hard- or soft-shelled gelatin type containing, for example, surfactants, lubricants, and inert fillers), tablets, lozenges (wherein the active substance is in a flavor, such as sucrose and acacia or tragacanth or the active substance is in an inert base, such as gelatin and glycerin), and troches, each containing a predetermined amount of IB-MECA or CL-IB-MECA as solids or granules; (c) powders; (d) suspensions in an appropriate liquid; (e) suitable emulsions; (f) liposome formulation; and others.

The invention will now be exemplified in the following description of experiments that were carried out in accordance with the invention. It is to be understood that these examples are intended to be in the nature of illustration rather than of limitation. Obviously, many modifications and variations of these examples are possible in light of the above teaching. It is therefore, to be understood that within the scope of the appended claims, the invention may be practiced otherwise, in a myriad of possible ways, than as specifically described hereinbelow.

BRIEF DESCRIPTION OF THE DRAWINGS

In order to understand the invention and to see how it may be carried out in practice, some preferred embodiments will now be described, by way of non-limiting examples only, with reference to the accompanying drawings, in which:

Fig 1A: shows the effect of PO administered IB-MECA 10 microgram/kg a day (square), or 100 microgram/kg a day (circle), or control untreated mice (diamond), on the inflammatory intensity in an animal model of adjuvant arthritis;

Fig. 1B: shows the effect of PO administered IB-MECA 1 microgram/kg a day (square), or control untreated mice (diamond), on the inflammatory intensity in an animal model of adjuvant arthritis;

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Figs 2A and 2B: show pictures of rats of Fig 1, having adjuvant induced arthritis, untreated (2A) and treated with 10 microgram/kg a day IB-MECA (treatment starts 7 days after disease induction (2B));

Figs 3A and 3B: show histological sections of a slice from a rat's joint 5 having adjuvant induced arthritis: untreated (3A) and treated with 10 microgram/kg a day IB-MECA, treatment starting 7 days after disease induction (3B);

Fig 4: shows inflammatory intensity in a model of adjuvant arthritis as a function of time for control untreated rats (dark circles); rats treated with 10 10 microgram/kg a day Cl-IB-MECA (squares) and rats treated with 100 microgram/kg a day of Cl-IB-MECA (triangles); treatment started 7 days after disease induction;

Figs 5A to 5D: shows pictures of rats (5A & 5C) and an enlarged picture of their paws (5B & 5D, respectively), having adjuvant induced arthritis treated (5A) 15 and untreated (5C) with CL-IB-MECA 10 microgram/kg a day, treatment starting 7 days after disease induction;

Fig. 6: shows the effect of administration of 10 microgram/kg of IB-MECA (CF-101) on joint swelling in a collagen-induced-arthritis (CIA) model in mice;

Fig. 7A: shows a histological section of joints of normal mice;

20 **Fig. 7B:** shows histological cross section of joints of untreated collagen-induced-arthritic mice;

Fig. 7C: shows histological cross section of collagen-induced-arthritic mice treated with 10 µg/kg IB-MECA;

Fig. 8: shows a combined histological score of CIA mice not treated 25 (control) and CIA mice treated with 10 microgram/kg of IB-MECA;

Fig. 9: shows the change in semi-recumbent heart rate as a function of plasma IB-MECA concentration after single doses of IB-MECA; and

Figs. 10A and 10B: show the change in standing heart rate as a function of plasma IB-MECA concentration after repeated doses of IB –MECA.

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DETAILED DESCRIPTION OF THE INVENTION

5 EXAMPLE 1: EXPERIMENTAL PROCEDURES

Materials and Methods

IB-MECA and Cl-IB-MECA were purchased from RBI/Sigma (Natick, MA, USA). For both reagents, a stock solution of 10 μ M was prepared in DMSO and
10 further dilutions in RPMI medium were performed.

1. Adjuvant Arthritis-Induction

Animal: Rats: LEWIS. Age: 9-12 Weeks. Sex: Female

Disease induction: 10 microgram/ml Heat killed Mycobacterium tuberculosis
15 H37Ra (Difco, Detroit, MI, USA) present in /IFA=Incomplete Freund's Adjuvant (Sigma), were injected in an amount of 100microliter at the tail base, S.C. (Sub Cut) In accordance with the ref: Current Protocols in Immunology Ed. John E. Coligon,(1996) Unit 15.5.

20 2. Collagen-Induced-Arthritis

Male DBA mice, aged 10 weeks, were injected SC with 100 μ l of Type II collagen 200 μ g in complete Freund's adjuvant (CFA). On day 21 a booster injection of the same emulsion was administered. Mice were inspected daily for symptoms of clinical arthritis.

25

3. Arthritis Evaluation

The inflammatory intensity, presented as joint swelling, was measured by Caliper (Mitutoyo Co., Tokyo, Japan). Histopathological sections of these decalcified whole joints were stained with hematoxylin-eosin. Slides were screened for the

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following arthritic characteristics: inflammatory cell infiltration, synovial cell lining hyperplasia and pannus formation. The histological assessment of the knees inflammation intensity was divided to four parameters:

1. Lymphatic follicle like formation- graded 0-4
- 5 2. Inflammatory cells infiltration- graded 0-4
3. Synovial cells hyperplasia- graded 0-4
4. Pannus formation- graded 0-4

The arithmetic sum of these four parameters above all together designated-"Total Arthritic Score" (Goldenberg *et al.*, J. Rheumatol. 1: 5-11,1983). The white blood cell (WBC) count and the serum levels of the pro-inflammatory cytokine TNF- α served as humoral markers of the immune system activation. Tumor necrosis factor- α sera level was determined, in accordance with the manufacturer's guidelines, by ELISA kit "Quantikine M" (R&D Systems, Minneapolis, MN, USA).

15

4. Statistical Analysis

The results were statistically evaluated using the Student's t-test. Comparison between the mean values of different experiments was carried out. The criterion for statistical significance was $p < 0.05$.

20

Example 1A: Effect of 10 and 100 $\mu\text{g/kg}$ IB-MECA on inflammatory intensity- adjuvant model

Rats were induced with adjuvant arthritis as described above. Animals were divided into three groups each consisting of 10 rats. Group 1 served as control while group 2 was administered with 100 $\mu\text{g/kg}$ PO IB-MECA a day, and group 3 was administered with 10 $\mu\text{g/kg}$ PO IB-MECA a day. Treatment with the active substance began 7 days after disease induction. The results are shown in Fig 1A. As

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can be seen, administration of 100 $\mu\text{g/kg}$ or 10 $\mu\text{g/kg}$ a day was effective in abolishing almost completely the inflammation as assessed by the inflammation intensity score. Fig. 1B shows the same experiment repeated with 1 $\mu\text{g/day}$. As can be seen even in this low dose a significant reduction was evident. Figs. 2A and 2B show pictures of a control untreated rat induced with adjuvant arthritis (2A) and a rat treated with 10 $\mu\text{g/kg}$ a day IB-MECA (2B). As can be seen while the paws of the untreated rat appear extremely swollen and red, the treated animal featured normal appearing paws.

Figs 3A and 3B show histological pictures of the joint of an untreated rat (3A), featuring the typical arthritis destruction of the synovial tissue and bone. Against this the histological picture (3B) of adjuvant arthritis induced rat treated with 10 $\mu\text{g/kg}$ a day of IB-MECA appeared completely normal without featuring any destructive processes.

15 **Example 1B: Effect of 10 and 100 $\mu\text{g/kg}$ Cl-IB-MECA on arthritis score – adjuvant model**

Rats were induced with adjuvant arthritis as described above. Rats were divided to three groups each consisting of 10 animals. Group 1 served as control while group 2 was administered with 100 $\mu\text{g/kg}$ PO Cl-IB-MECA a day, and group 3 was administered with 10 $\mu\text{g/kg}$ CL-IB-MECA PO a day. Administration was initiated at day 7 after disease induction. The results are shown in Fig 4.

As can be seen administration of 10 $\mu\text{g/kg}$ a day was significantly more effective in improving the arthritis score than administration of 100 $\mu\text{g/kg}$ a day indicating that surprisingly the active ingredient does not have a classic “dose response effect” wherein the higher the dosage the more pronounced the effect, but rather a bell-shaped curve effect, where increase in the dosage caused decrease in the therapeutical effect. Figs 5A to 5D show photographs of a control untreated rat induced with adjuvant arthritis (5A) and a rat treated with 10 $\mu\text{g/kg}$ a day-CL-IB-

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MECA (5C). While the paws of the untreated rat appear extremely swollen and red, the treated animal featured normal appearing paws.

5 **Example 1C: Effect of 10 µg/kg IB-MECA on joint swelling and histology score-collagen induced model in mice**

Male DBA mice were treated to produce collagen-induced-arthritis as described above. Each group contained 10 animals and each experiment was conducted at least three times.

10 IB-MECA (10 µg/kg) was orally administered by gavage, twice daily starting at onset of clinical arthritis. The positive control received vehicle only. The inflammatory intensity in the CIA model was determined in accordance with the increase in the mice hind paw's diameter, measured by caliper (Mitotoyo, Tokyo, Japan). The mean score in each experimental group was designated as the "Clinical
15 Score".

Histology Score

Animals were sacrificed, the legs were removed up to the knees level, fixed in 10% formaldehyde, decalcified, dehydrated, paraffin-embedded, cut into 4 µm sections and stained by Hematoxylin-Eosin.

20 The assessment of all pathologic findings were performed blind (by L.R-W and M.H) using semi-quantitative grading scales of 0 to 4 for the following parameters:

- a) The extent of inflammatory cells' infiltration to the joint tissues,
- b) Synovial lining cell hyperplasia,
- 25 c) Pannus formation,
- d) Joint cartilage layers destruction,
- e) Bone damage and erosion score was graded 0-5: 0-normal.

1- minimal loss of cortical bone at a few sites; 2- mild loss of cortical trabecular bone; 3- moderate loss of bone at many sites; 4- marked loss of bone at

- 18 -

many sites; 5-marked loss of bone at many sites with fragmenting and full thickness penetration of inflammatory process or pannus into the cortical bone (17,18). The mean of all the histological parameters scores were designated "Histology Score".

5 Results

In the following experiments, the effect of IB-MECA (10 µg/kg/day) on the development of collagen-induced arthritis was evaluated. The mice developed the disease several days after the second immunization with Type II collagen/CFA emulsion. In each individual mouse, IB-MECA was administered at the onset of
10 disease. In the control group a maximal hind paw swelling was observed 6 days after disease onset, whereas in the IB-MECA treated group it was noted on day 14th (Fig. 6). The intensity of the arthritis was reduced throughout the experimental period, but on day 21 this difference became statistically significant ($p < 0.02$). In the IB-MECA treated group (Fig. 7C) histology sections showed minimal
15 inflammatory changes compared to the extensive one in the control group (Fig. 7A) and a pattern that was similar to control (Fig. 7B). In addition, the histology score was markedly lower in the IB-MECA treated group compared to the control group (3.87 ± 1.2 vs. 1.0 ± 0.6 , $p < 0.01$) (Fig. 8).

20 Example 1D: Clinical Trial in humans – effect of IB-MECA on rheumatoid arthritis

Patients with active rheumatoid arthritis were chosen for the study. All chosen patients met the following criteria:

25

Inclusion Criteria

Rheumatoid arthritis patients that were included in the study, met the following inclusion criteria:

1. Males and females 18 years of age or older

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2. Functional Class I, II, or III by the criteria of the American College of Rheumatology
3. Active RA, as indicated by the presence of (a) ≥ 6 swollen joints; AND (b) ≥ 9 tender joints; AND at least one of the following: (c) Westergren ESR of ≥ 28 mm/hour; OR (d) CRP level of > 2.0 mg/dL; OR (e) morning stiffness for ≥ 45 minutes
4. History of unsuccessful treatment (documented intolerance or lack of efficacy as determined by the Investigator) with at least 1, but no more than 4, of the following disease modifying anti-rheumatic drugs (DMARDs): methotrexate, hydroxychloroquine, sulfasalazine, oral or injectable gold, azathioprine, leflunomide, minocycline, and penicillamine, alone or in combination

Protocol

Patients included in the study were subjected to a 1 month washout period in which they ceased to take any DMARD. Following this one month washout the patients were administered twice daily with either 0.1 mg, 1 mg or 4 mg of IB-MECA in a double-blind fashion. The drug was formulated in a soft gel capsule containing IB-MECA dissolved in Cremophor RH. The patients received the drug for a period of up to 12 weeks.

Clinical Assessment

Disease activity was assessed using standard criteria as laid down by the American College of Rheumatology (ACR).

Results

15 patients were treated and in at least half of them a significant improvement in disease symptoms was observed for a period of up to the 12 weeks of the study.

- 20 -

EXAMPLE 2: DETERMINING MAXIMAL TOLERATED DOSE OF IB-MECA IN HUMANS

Methods

Study design

5 Two clinical studies were carried out: a single dose study and a repeat dose study. Both studies were parallel-group, double-blind, dose-rising, and placebo-controlled in design.

In the single dose study, 15 healthy men (3 groups of 5) received a single oral dose of IB-MECA (1, 5 or 10 mg) or placebo. In each group, 1 subject
10 received placebo. In the repeated dose study, 28 healthy men (4 groups of 7) received repeated oral doses of IB-MECA (2, 3, 4 or 5 mg) or placebo every 12 h for 7 days. In each group, 2 subjects received placebo.

Selection of Subjects

Healthy young men, aged 18–45 years.

15 *Study drugs*

In the single dose study, a solution with IB-MECA powder in 30% Cremophor RH40 (BASF) was used. In the repeated dose study, an aqueous 0.5% methylcellulose suspension (Methocel A4M Premium, The Dow Chemical Company) was used. In both studies, the study medication was taken orally as a
20 drink, followed by 50 ml of tap water.

Study procedures

The following procedures were done:

- Safety assesment: laboratory assessments (routine biochemistry and urinalysis), physical examination, 12-lead ECG, ambulatory ECG, pulmonary
25 function testing (FEV1), vital signs (semi-recumbent in the single dose study; semi-recumbent and standing in the repeat dose study). Adverse events were recorded throughout both studies.
- Determination of IB-MECA blood level: in the single dose study blood samples for assay of IB-MECA were taken immediately before and at 0.25,

– 21 –

0.5, 1, 2, 4, 8, 12, 24, and 48 h after dosing; in the repeat dose study – blood samples were taken immediately before and at 0.25, 0.5, 1, 2, 4, 8, and 12 h after dosing on Day 1, before dosing on Days 2–6, and before and at 0.25, 0.5, 1, 2, 4, 8, 12, and 24 h after dosing on Day 7.

- 5 Plasma samples were assayed for IB-MECA using LC/MS/MS. The lower limit of quantification (LLOQ) was 0.1 ng/mL. Intra-assay coefficients of variation (CV) were <5.0% and inter-assay CVs were <9.4%.

Pharmacokinetic analysis

- Maximum concentration (C_{\max}) and time to maximum concentration (t_{\max}) were observed values. Other pharmacokinetic parameters (half-life, $t_{1/2}$; AUC; and clearance, CL/F) were calculated by non-compartmental methods using WinNonlin® software (version 3.0, Pharsight, Mountain View, CA, US). Accumulation indices of C_{\max} and AUC were calculated as ratio of values at steady state (Day 7) to the values on Day 1.

15 ***Statistical analysis***

- Data from all subjects who received IB-MECA were included in the analysis of safety and tolerability (adverse events and laboratory safety variables). Numerical data and parameters were summarised using means or medians, and other descriptive statistics, according to the type and distribution of the data.

Results

Study population

- In the single dose study, the mean (range) age, weight, and height were 28.3 (20–40 years, 75.9 (63–98) kg, and 177.8 (167–188) cm, respectively. In the repeat dose study, the mean (range) age, weight, and height were 25.2 (18–45) years, 75.3 (56–99) kg, and 178.0 (163–189) cm, respectively. All volunteers were of Europid ethnic origin, except for 2 Asian/Indian men and 1 Europid/Oriental man.

All subjects were deemed healthy at screening, without any

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haematological disorder or history of splenectomy, nor splenomegaly on physical examination.

Safety and tolerability

In the single dose study, IB-MECA in doses up to 5 mg was well
5 tolerated, as judged by vital signs, physical examination, FEV1, and 12-lead and
continuous ECG. There were no clinically significant changes in safety tests of
blood and urine. Four subjects had a small increase in resting heart rate after 5
mg IB-MECA; however, after the 10 mg dose, 4 subjects had substantial
increases in resting heart rate, 2 of which were substantial (up to 115 beats/min)
10 and considered drug-related. Those 2 subjects developed nausea, and 1 of them
vomited once and was facially flushed. Those changes precluded our studying
higher doses. In no subject was there a significant change in blood pressure, but
blood pressure was not measured in the standing position. The increase in heart
rate was closely related to the plasma IB-MECA concentration (Fig. 9).

15 In the repeat dose study, IB-MECA had an acceptable safety profile, as
judged by vital signs, physical examination, FEV1, and 12-lead and continuous
ECG. There was a dose-related increase in heart rate on Day 1, but some
tolerance developed, because that effect was clearly smaller on Day 7. On Day 1,
the time course of the increase in heart rate reflected the profile of plasma IB-
20 MECA concentrations. However, on Day 7, equivalent plasma IB-MECA
concentrations were associated with smaller increases in heart rate (Figs. 10A &
10B). There were no clinically significant changes in safety tests of blood and
urine.

Most of the adverse events occurred during the 5 mg dose regimen:
25 headache and drowsiness were most common. Two adverse events were vascular
disorders – hot flushes and dizziness on standing.

Overall, IB-MECA was well tolerated at single doses of up to 5 mg and
repeat doses of up to 4 mg 12-hourly. Adverse events were related to dose and
generally occurred around the time of maximal blood concentration (t_{max}). After

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single doses of up to 5 mg IB-MECA, there were no adverse events within 12 h of dosing, but after a single dose of 10 mg, there were 8 adverse events within 12 h of dosing. After repeated doses of up to 4 mg 12-hourly, there were 2 adverse events within 12 h of dosing. However, after repeated doses of 5 mg 12-hourly, there were 13 adverse events within 12 h of dosing. Thus, based on this repeat dose study, the 4 mg dose was determined to be the maximum tolerated dose for a twice-daily therapeutic regimen.

Overall a single daily dose of 5 mg and a twice daily dose of 4 mg were considered safe and well tolerated. Given the fact that these doses gave a plasma level (C_{max}) of less than 160 nM (80 ng/ml)

Pharmacokinetics

The pharmacokinetics of single doses of IB-MECA are shown in the following Table 1:

Table 1
Mean (SD) plasma PK parameters after a single oral dose of CF101 (n=4 per group)

Dose (mg)	C_{max} (ng/mL)	t_{max} ^a (h)	$AUC_{(0-48)}$ (ng•h/mL)	$AUC_{(inf)}$ (ng•h/mL)	$t_{1/2}$ (h)	CL/F (L/h)
1	21.2 (2.1)	1 (1-2)	220.7 (20.9)	225.2 (21.7)	8.7 (0.7)	4.5 (0.4)
5	81.6 (23.6)	1 (1-2)	872.3 (211.6)	904.0 (221.9)	8.3 (0.2)	5.8 (1.4)
10	178.0 (46.6)	1 (1-2)	1780.0 (228.7)	1813.0 (226.5)	8.6 (0.4)	5.6 (0.7)
a median (range)						

As can be seen, IB-MECA pharmacokinetics were linear, and inter-subject variability was low. IB-MECA was absorbed rapidly: t_{max} ranged between 1-2 h. Mean C_{max} (maximal plasma level) and AUC_{0-48} (area under the curve of blood level over 48 hours after administration) were related to dose. C_{max} was 21.2, 81.6, and 178.0 ng/ml, and AUC_{0-48} was 220.7, 872.3, and 1780.0 ng.h/ml, for doses of 1, 5, and 10 mg, respectively. The half-life of about 8.5 h was independent of dose. Apparent plasma clearance (CL/F) was low (4-7 L/h) and independent of dose.

The pharmacokinetics of repeated doses of IB-MECA are shown in the following Table 2:

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Table 2
Mean (SD) plasma PK parameters on Days 1 and 7 of repeated dosing with
CF101 (n=5 per group)

5

Dose (mg)	Day	C _{max} (ng/mL)	t _{max} ^a (h)	AUC ₍₀₋₁₂₎ (ng•h/mL)	AUC ^b (ng•h/mL)	t _{1/2} (h)	CL/F (L/h)
2	1	22.0 (3.3)	2 (1-4)	155.2 (39.1)	207.4 (52.0)	5.52 (0.2)	10.1 (2.3)
	7	30.9 (3.1)	2 (1-2)	242.4 (41.4)	346.3 (64.0)	9.83 (1.2)	4.9 (0.7)
3	1	49.3 (9.7)	2 (1-2)	304.5 (19.5)	423.5 (27.5)	6.29 (1.0)	7.1 (0.5)
	7	49.0 (7.9)	1 (1-2)	341.6 (38.1)	512.1 (74.1)	9.25 (0.8)	5.0 (0.6)
4	1	46.2 (11.4)	2 (1-2)	297.0 (57.2)	400.2 (85.7)	5.77 (0.6)	10.4 (2.1)
	7	58.1 (10.4)	1 (1-2)	458.3 (54.8)	640.3 (73.7)	8.93 (0.8)	5.4 (0.7)
5	1	63.6 (22.0)	2 (1-2)	461.6 (157.4)	596.1 (196.6)	4.96 (0.3)	9.3 (3.5)
	7	79.5 (24.1)	2 (2-2)	601.0 (163.6)	818.4 (214.0)	9.39 (0.6)	5.4 (1.5)
		a median (range)					
		b AUC _(inf) on Day 1, AUC ₍₀₋₂₄₎ on Day 7					

IB-MECA was absorbed rapidly: t_{max} was 1-2 h. Steady state was
 10 reached by Day 3. IB-MECA pharmacokinetics did not change after repeated
 dosing. Plasma concentrations of IB-MECA were dose proportional on Day 1
 and at steady state (Day 7). Half-life of IB-MECA was independent of dose, and
 was about 9-10 h at steady state. As in the single dose study, apparent plasma
 clearance (CL/F) was low (5-10 L/h) and independent of dose. The
 15 accumulation indices ranged between 1-1.4 and 1.1-1.6 for C_{max} and AUC,
 respectively; the accumulation indices were much as predicted from the single
 dose data.

Summary

Overall a single daily dose of 5 mg and a twice daily dose of 4 mg where
 20 considered safe and well tolerated. Given the fact that. These doses gave a C_{max}
 of less than about 160 nM (80 ng/ml).

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CLAIMS:

1. A method for the treatment of inflammatory arthritis (IA) in a human subject, comprising: orally administering to an individual in need of such treatment an effective amount of N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-
5 MECA) or 2-chloro-N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (Cl-IB-MECA;
wherein the effective amount is an amount which is in a range between D_X and D_Y; D_X being an amount lower than D_{MAX} and D_Y being an amount higher than D_{MAX}; D_{MAX} being an amount that yields a maximal therapeutic effect; both D_X and
10 D_Y yield a therapeutic effect that is substantially less than that obtained at D_{MAX}.
2. A method according to Claim 1, wherein the effective amount is less than the maximal tolerated dose.
3. A method according to claim 1, wherein the effective amount is such so as to yield a therapeutic effect that is at least 50% of the maximal therapeutic effect that
15 can be achieved with said active agent.
4. A method of Claim 1, wherein the active agent is administered once a day at a dose of less than about 70 microgram/Kg.
5. A method of Claim 1, wherein the active agent is administered once a day at a dose of less than about 5 mg.
- 20 6. A method of Claim 1, wherein the active agent is administered twice a day at a dose of less than about 57 microgram/Kg each dose
7. A method of Claim 1, wherein the active agent is administered twice a day at a dose of less than about 4 mg each dose.
8. A method for the treatment of inflammatory arthritis (IA) in a human
25 subje^tc, comprising orally administering to an individual in need of such treatment an effective amount of N⁶-(3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (CL-IB-MECA), wherein the effective amount is an amount which the human

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equivalent of a murine dose of 0.001 mg/Kg to 0.4 mg/Kg administered once or twice per day

9. A method according to Claim 7, wherein the effective amount is an amount within the range of about 0.14 to about 57 microgram/Kg.
- 5 10. A method according to Claim 7, wherein the effective amount is within the range of about 0.01 to 4 mg.
11. A method according to Claim 7, wherein the effective amount is an amount within the range of about 0.14 to about 28 microgram/Kg.
12. A method according to Claim 7, wherein the effective amount is a dose
10 within the range of about 0.01 to 2 mg.
13. A method according to Claim 7, wherein the effective amount is an amount within the range of about 1.4 to about 21 microgram/Kg.
14. A method according to Claim 7, wherein the effective amount is a dose within the range of about 0.1 to 1.5 mg.
- 15 15. Use of an effective amount of N6- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (Cl-IB-MECA) for the preparation of a pharmaceutical composition for the treatment of inflammatory arthritis, the effective amount is an amount which is in a range between D_X and D_Y ; D_X being an amount lower than D_{MAX} and D_Y being
20 an amount higher than D_{MAX} ; D_{MAX} being an amount that yields a maximal therapeutic effect; both D_X and D_Y yield a therapeutic effect that is substantially less than that obtained at D_{MAX} .
16. Use according to Claim 15, wherein the effective amount is less than the maximal tolerated dose.
- 25 17. Use according to Claim 15, for the production of a pharmaceutical composition for once daily administration, wherein the effective amount is an amount for administration to a human individual at a dose of less than about 70 micrograms/Kg.

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18. Use according to Claim 15, for the production of a pharmaceutical composition for once daily administration, wherein the effective amount is less than about 5 mg.
19. Use according to Claim 15, for the production of a pharmaceutical
5 composition for twice daily administration, wherein the effective amount is an amount for administration to a human individual at a dose of less than about 57 micrograms/Kg.
20. Use of an effective amount of N6- (3-iodobenzyl)-adenosine- 5'-N-methyl-uronamide (IB-MECA) or 2-chloro-N⁶- (3-iodobenzyl)-adenosine- 5'-N-methyl-
10 uronamide (Cl-IB-MECA) for the preparation of a pharmaceutical composition for the treatment of inflammatory arthritis, the effective amount is an amount which the human equivalent of a murine dose of 0.001 mg/Kg to 0.4 mg/Kg administered once or more per day.
21. Use according to Claim 20, for the production of a pharmaceutical
15 composition for twice daily administration.
22. Use according to Claim 20, for the production of a pharmaceutical composition for administering said active agent to a subject a dose of about 0.14 to about 57 microgram/Kg.
23. Use according to Claim 20, wherein the effective amount is within the range
20 of about 0.01 to 4 mg.
24. Use according to Claim 20, for the production of a pharmaceutical composition for administering said active agent to a subject a dose of about 0.14 to about 28 microgram/Kg.
25. Use according to Claim 19, wherein the effective amount is within the range
25 of about 0.01 to 2 mg.
26. Use according to Claim 20, for the production of a pharmaceutical composition for administering said active agent to a subject a dose of about 1.4 to about 21 microgram/Kg.

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27. Use according to Claim 20, wherein the effective amount is within the range of about 0.1 to 1.5 mg.
28. A pharmaceutical composition for use in the treatment of a method according to any one of claims 1 to 14.

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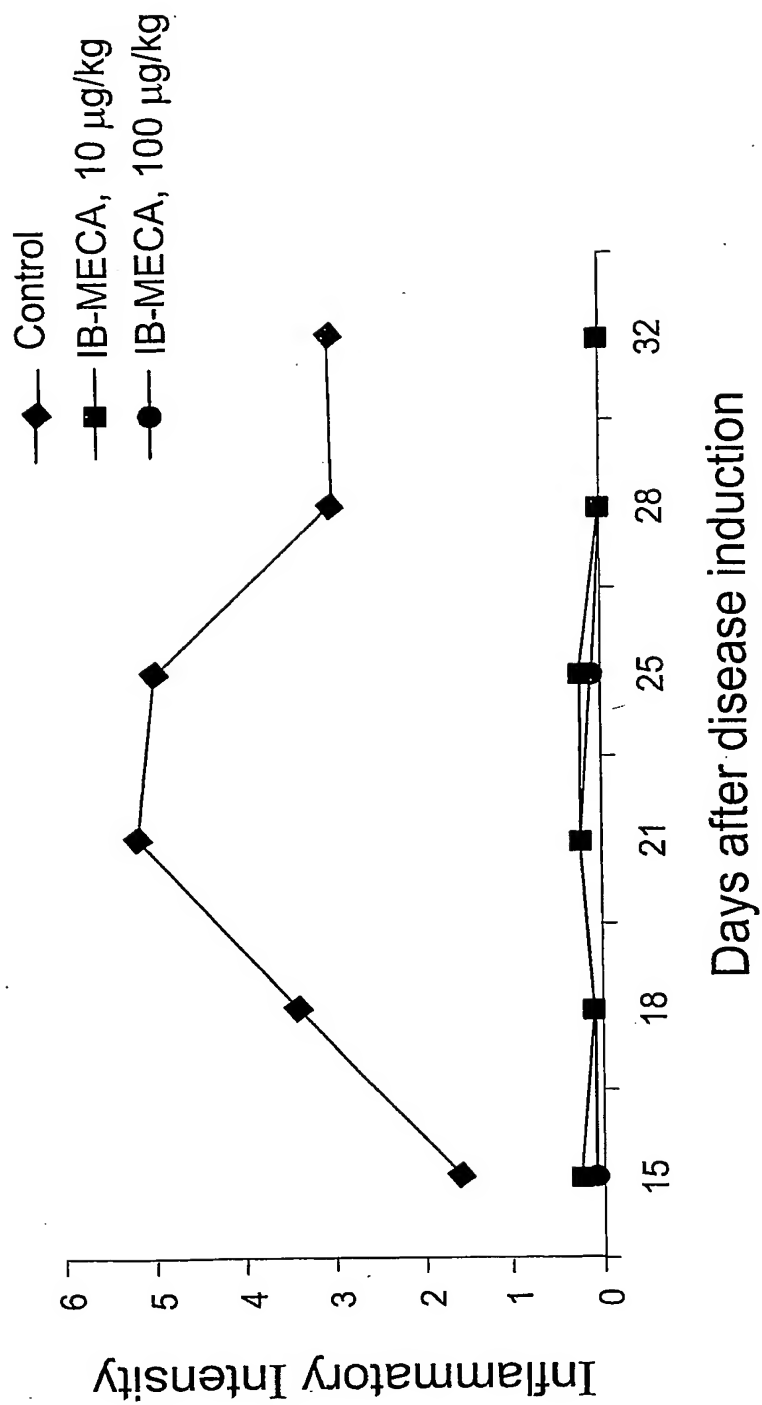


FIG. 1A

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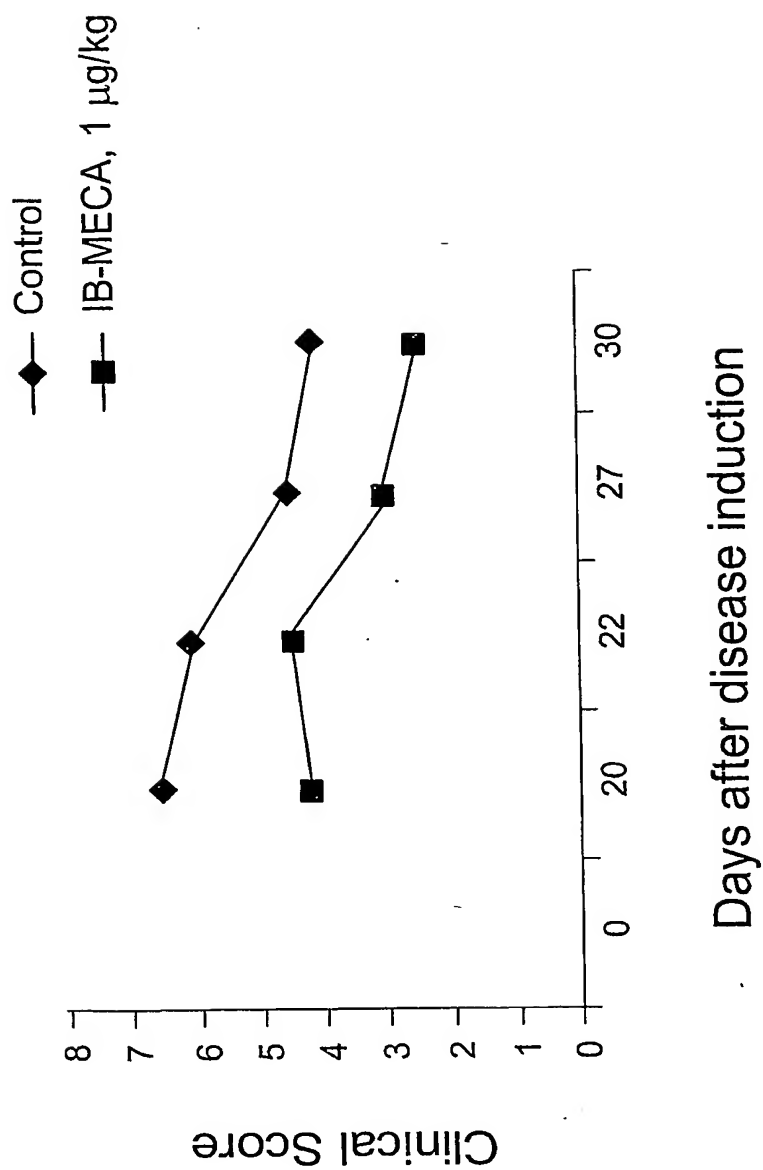


FIG. 1B

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FIG. 2B

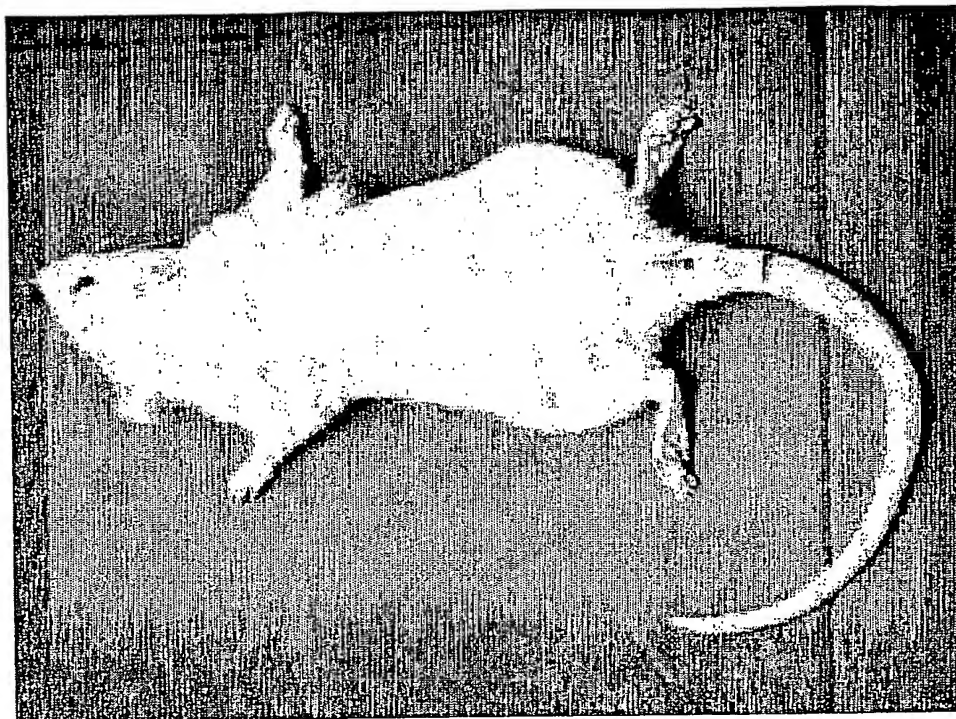
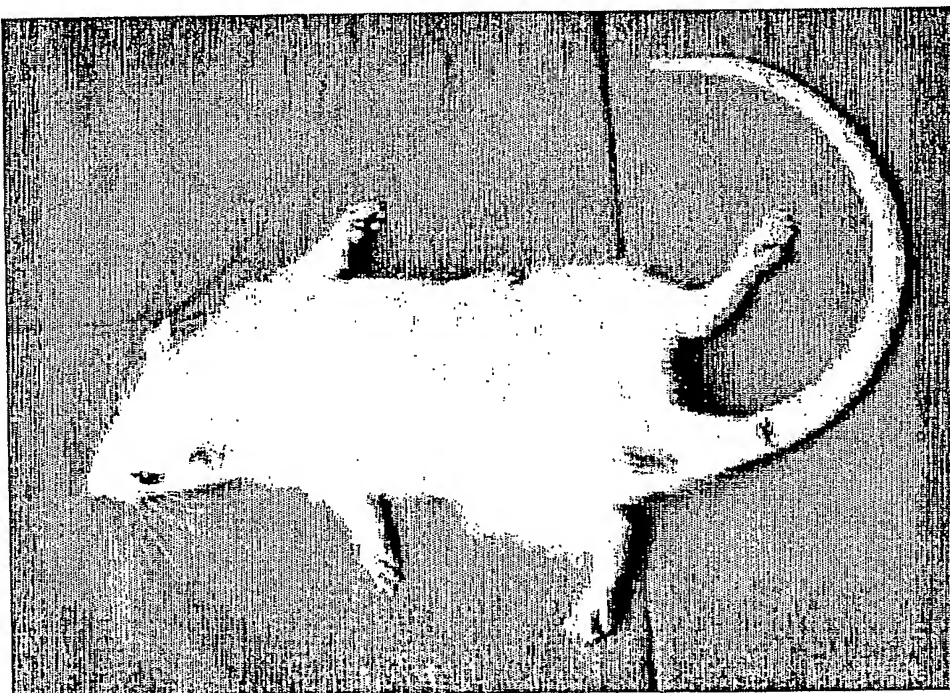


FIG. 2A



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FIG. 3B **IB-MECA**

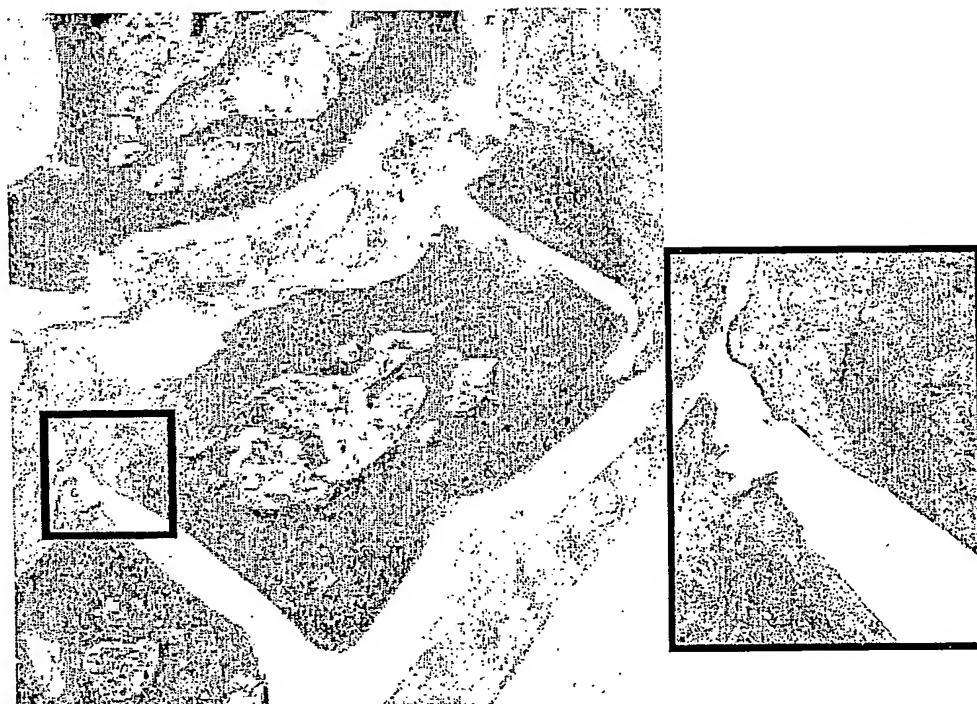
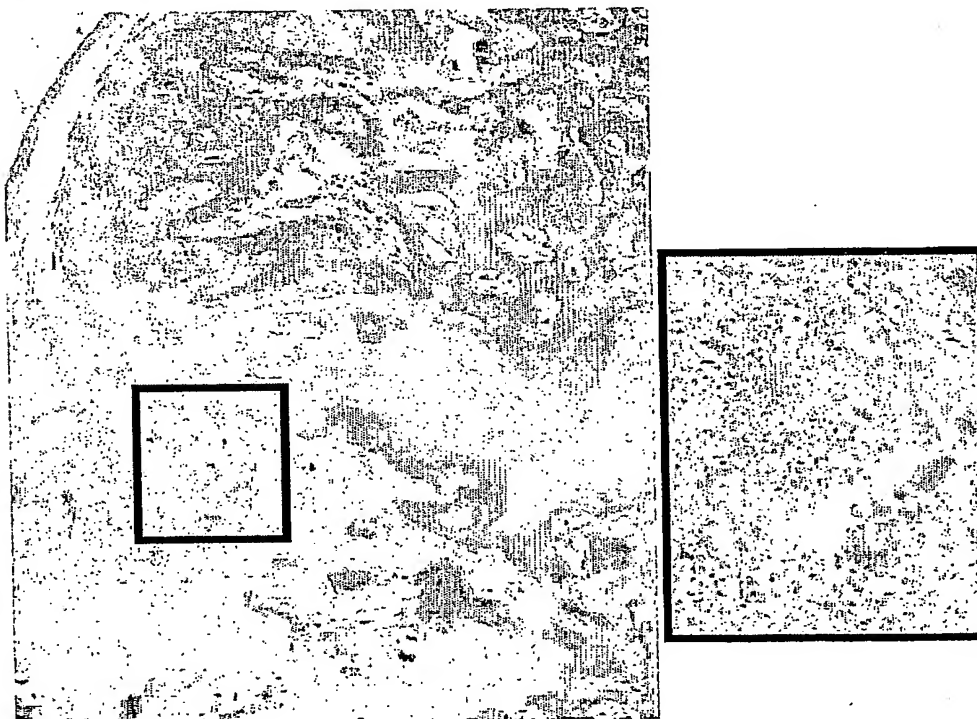


FIG. 3A **Untreated**



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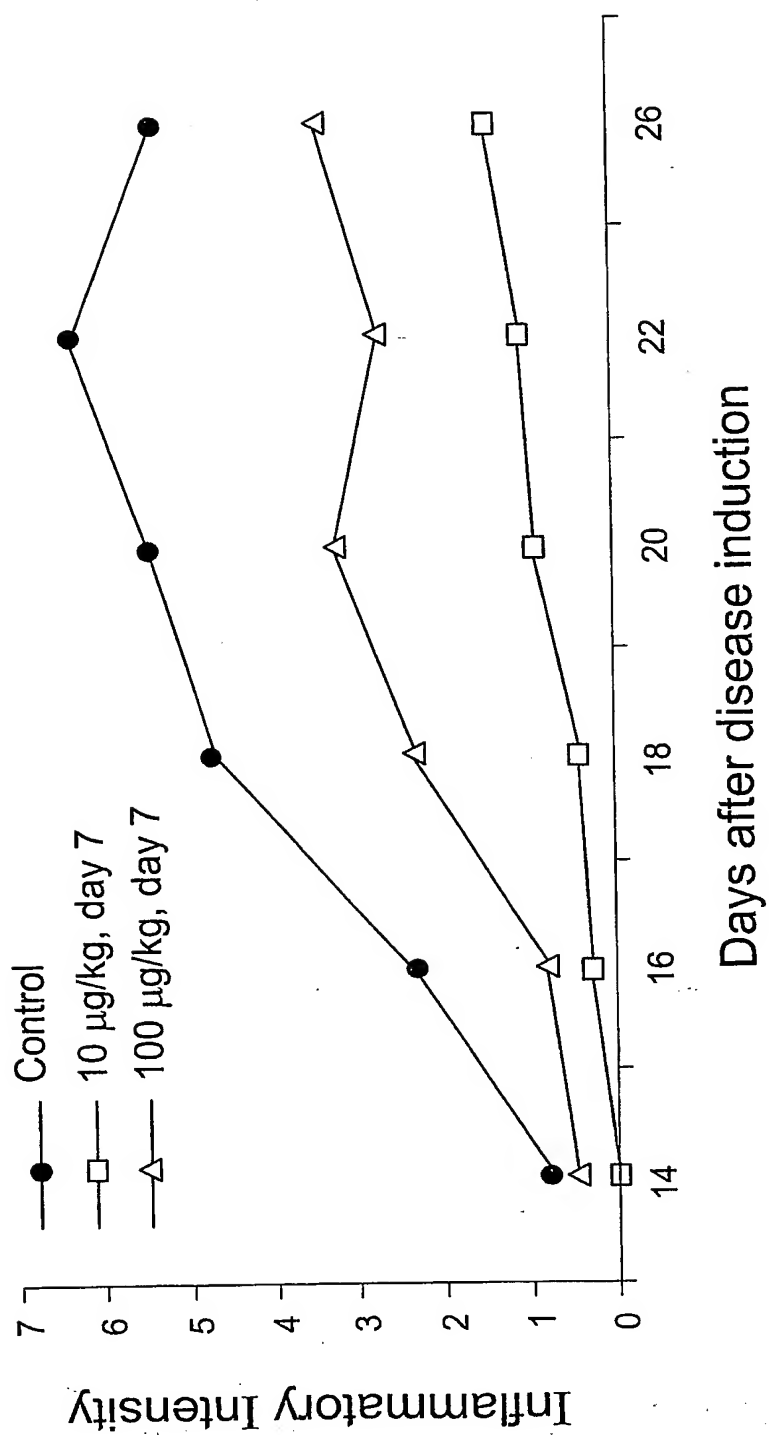
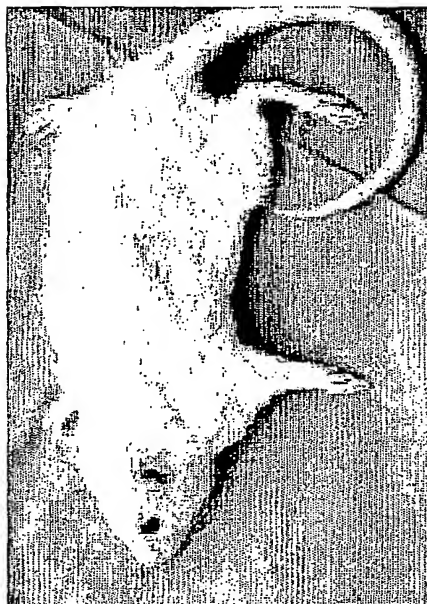


FIG. 4

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CI-IB-MECA

FIG. 5C



Untreated

FIG. 5A



FIG. 5D



FIG. 5B

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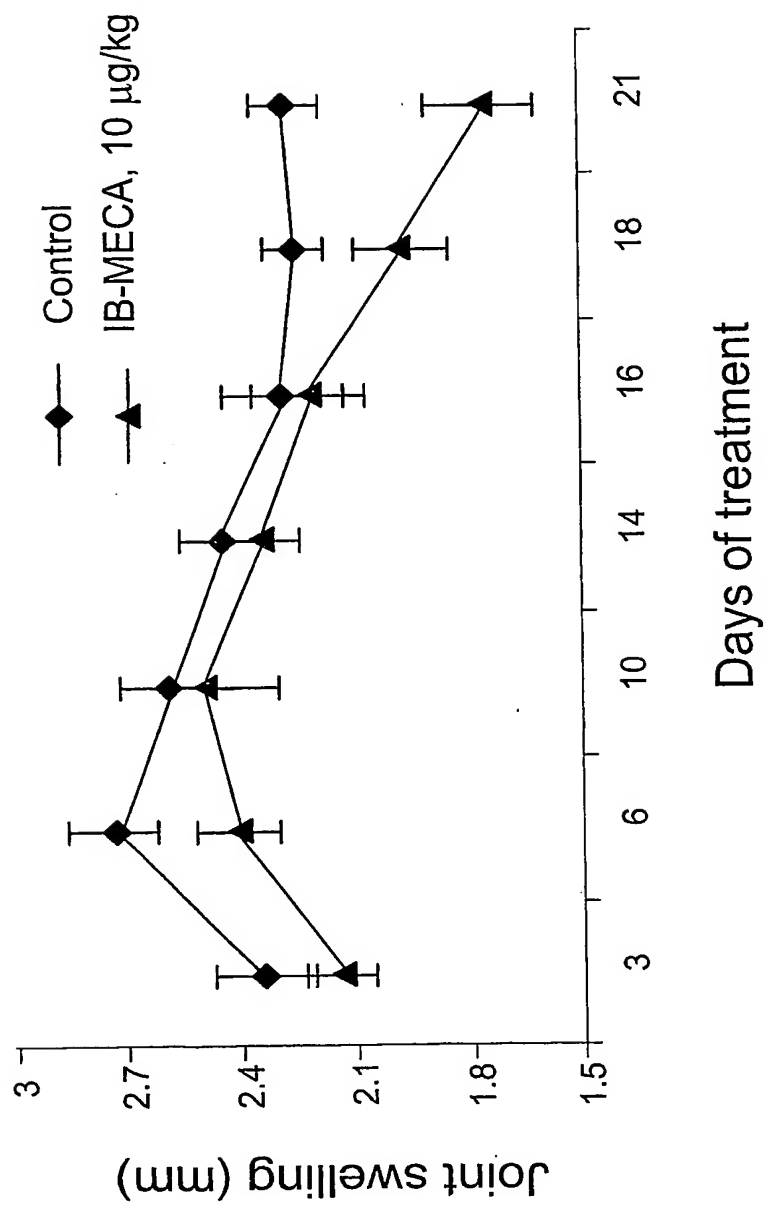


FIG. 6

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IB - MECA



FIG. 7C

Control



FIG. 7B

Naive



FIG. 7A

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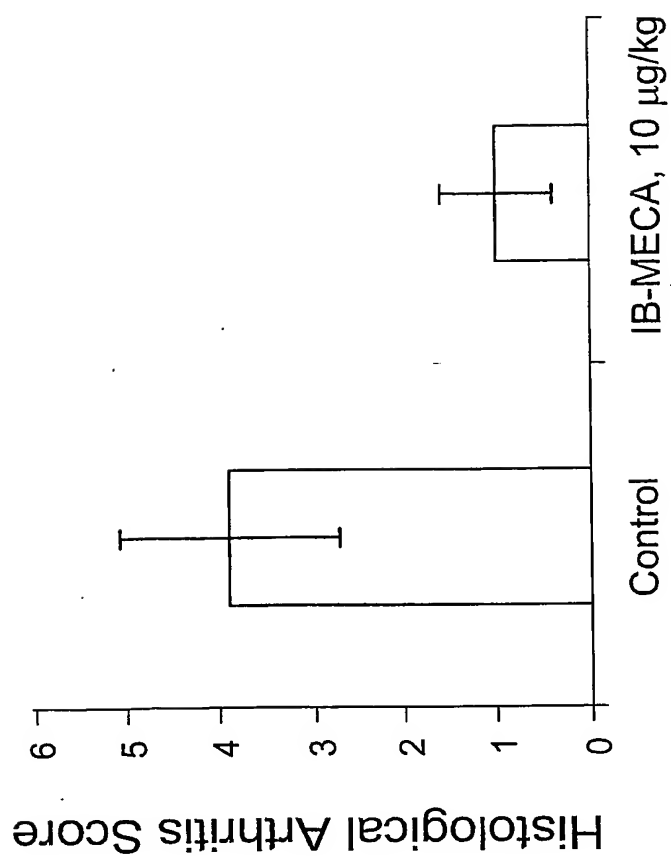


FIG. 8

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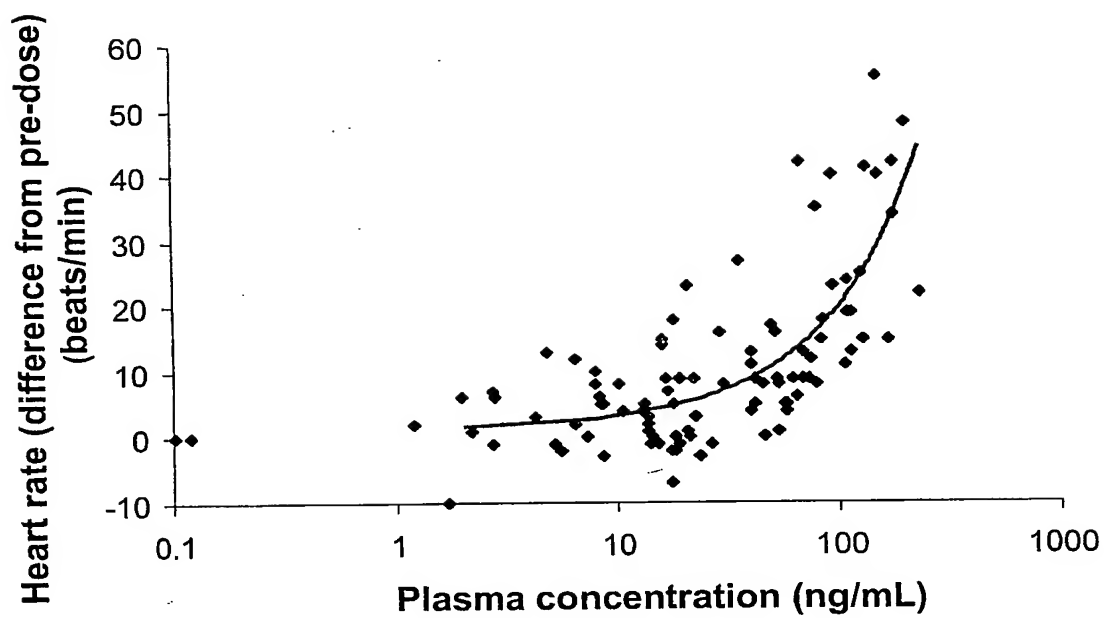


FIG. 9

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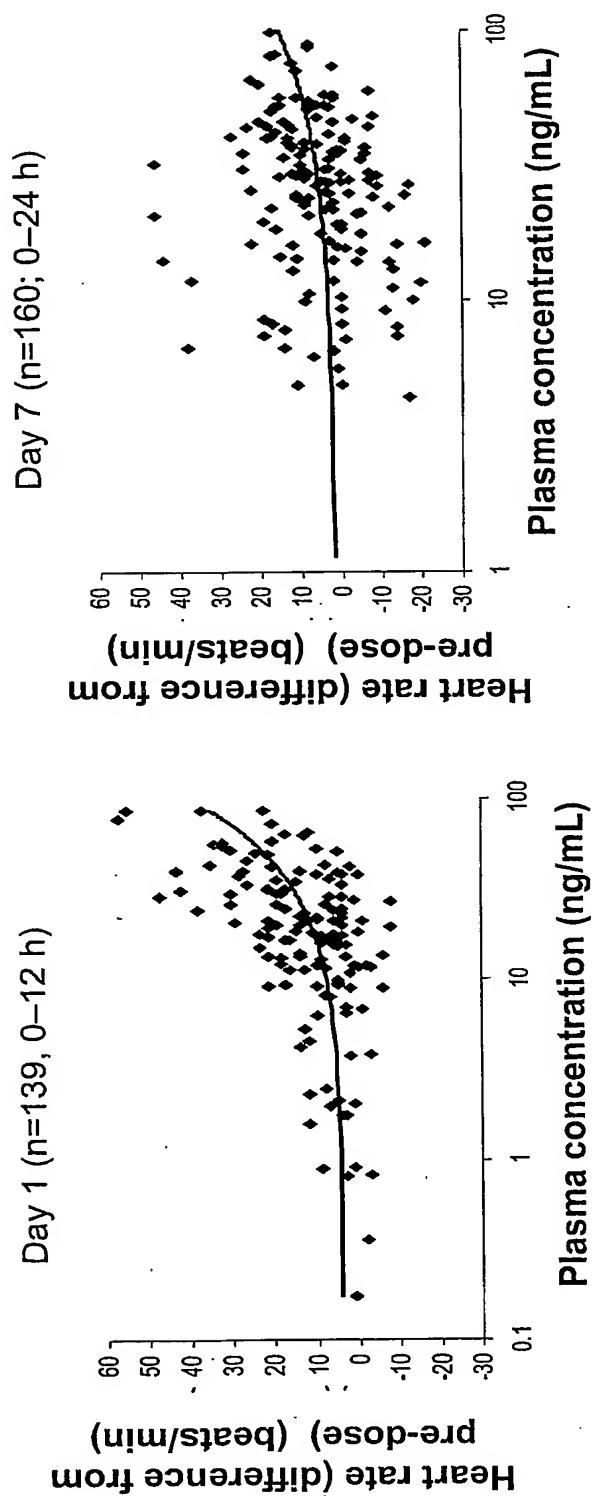


FIG. 10B

FIG. 10A

INTERNATIONAL SEARCH REPORT

International Application No

PCT/IL 03/00981

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/7076 A61P19/02

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, BIOSIS, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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P, X

BAHARAV EHUD ET AL: "The anti-inflammatory effect of A3 adenosine receptor agonists in murine autoimmune arthritis models."
INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE,
vol. 12, no. Supplement 1, 2003, page S42, XP0009026426
& 8TH WORLD CONGRESS ON ADVANCES IN ONCOLOGY AND 6TH INTERNATIONAL SYMPOSIUM ON MOLECULAR MEDICINE; CRETE, GREECE;
OCTOBER 16-18, 2003
ISSN: 1107-3756
abstract

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1-28

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
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Date of the actual completion of the international search

26 February 2004

Date of mailing of the international search report

04/03/2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Skjöldebrand, C

INTERNATIONAL SEARCH REPORT

Internatio application No

PCT/IL 03/00981

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 773 423 A (GALLO-RODRIGUEZ CAROLA ET AL) 30 June 1998 (1998-06-30) cited in the application column 25, line 40 claims	1-28
X	BAHARAV EHUD ET AL: "The effect of adenosine and the A3 adenosine receptor agonist IB-MECA on joint inflammation and autoimmune diseases models" INTERNATIONAL JOURNAL OF MOLECULAR MEDICINE, vol. 10, no. Supplement 1, 2002, page S104, XP0009026428 & 7TH WORLD CONGRESS ON ADVANCES IN ONCOLOGY AND THE 5TH INTERNATIONAL SYMPOSIUM ON MOLECULAR MEDICINE; HERSONISSOS, CRETE, GREECE; OCTOBER 10-12, 2002 ISSN: 1107-3756 abstract	1-28
X	FANG G D ET AL: "Adenosine (ADO) agonists protect mice from death in a model of endotoxemia by binding to A2A but not A3 ADO receptors" ABSTRACTS OF THE INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 41, 2001, page 75, XP0001179637 & 41ST ANNUAL MEETING OF THE INTERSCIENCE CONFERENCE ON ANTIMICROBIAL AGENTS AND CHEMOTHERAPY; CHICAGO, ILLINOIS, USA; SEPTEMBER 22-25, 2001 abstract	1-28
X	MABLEY JON G ET AL: "The A3 adenosine agonist, IB-MECA, protects against the development of arthritis and reverses established arthritis" FASEB JOURNAL, vol. 16, no. 5, 22 March 2002 (2002-03-22), page A1044, XP0009026430 & ANNUAL MEETING OF PROFESSIONAL RESEARCH SCIENTISTS ON EXPERIMENTAL BIOLOGY; NEW ORLEANS, LOUISIANA, USA; APRIL 20-24, 2002 ISSN: 0892-6638 abstract	1-28

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INTERNATIONAL SEARCH REPORT

Internatio
pplication No
PCT/IL 03/00981

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SZABO CSABA ET AL: "Suppression of macrophage inflammatory protein (MIP)-1alpha production and collagen-induced arthritis by adenosine receptor agonists" BRITISH JOURNAL OF PHARMACOLOGY, vol. 125, no. 2, September 1998 (1998-09), pages 379-387, XP0001179576 & ISSN: 0007-1188 cited in the application abstract	1-28
X	HASKO G ET AL: "Stimulation of adenosine 3 receptors exerts anti-inflammatory effects in acute and chronic models of inflammation" DRUG DEVELOPMENT RESEARCH, vol. 43, no. 1, January 1998 (1998-01), page 39, XP0009026431 & 6TH INTERNATIONAL SYMPOSIUM ON ADENOSINE AND ADENINE NUCLEOTIDES: NEW FRONTIERS IN THE 3RD MILLENNIU; FERRARA, ITALY; MAY 19-24, 1998 ISSN: 0272-4391 abstract	1-28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IL 03/00981

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 1-14 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

Information on patent family members

Application No

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
US 5773423	A	30-06-1998	US 5688774 A	18-11-1997
			AT 206432 T	15-10-2001
			AU 7331094 A	13-02-1995
			DE 69428536 D1	08-11-2001
			DE 69428536 T2	06-06-2002
			EP 0708781 A1	01-05-1996
			WO 9502604 A1	26-01-1995